DEVELOPMENT OF NANOFIBROUS MEMBRANES TOWARDS BIOLOGICAL SENSING

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ABSTRACT

Homeland Security and the Veterinary Services Activity, Office Of The Surgeon General (VSA, OTSG) have identified food as potential instruments for covert acts of bioterrorism involving Chem/Bio agents. Therefore, VSA, OTSG has established the need to develop technologies that can be used in the presumptive screening of food to minimize the health effects on the warfighter. Currently, there are no deployable biological detection capabilities that are truly rapid when sample preparation is included in the detection process. The complexity of food matrices makes its very difficult to analyze directly by modern day PCR and immunoassays systems, no matter how sensitive, without first diluting and removing interfering substances inherent to the food products. The result is that the concentration of the biological agent introduced into the analyzer may be so dilute that the instrument cannot detect it even though its presences is at concentrations that can cause human illness or death. This is why the testing of bacterial agents from food usually requires a 24-hour enrichment step, which defeats the purpose of rapid detection built into these new biosensor type technologies. Also, biological toxins, as opposed to live agents, cannot be replicated by enrichment. Dilution of toxins from a food matrix may produce a false negative test delaying the actual identification of the toxin until after the effects have already occurred.

There has been tremendous progress in the development of rapid detection technologies, but very little has been accomplished in the development of novel methods for capturing and concentrating biological agents from complex matrices. Other than magnetic beads, current practices in sampling and preparation vary slightly from what has been traditionally practiced for standard plate culturing. Rapid detection of biological agents from complex food matrices requires both dilution and removal of assay inhibitory substances. Development of new approaches towards improving sample preparation and capture efficiency should provide a means for real-time identification of low lethal and sublethal levels of biological agents from food. Our approach to improve sample preparation for complex matrices was to generate high surface area nanofibrous membranes with covalently attached molecular recognition elements (MREs, e.g. antibodies, peptides/DNA) for the selective binding/capture of target biological agents through the use of electrospinning techniques. Electrospinning is a process by which high static voltages are used to produce an interconnected membrane-like web of small fibers with diameters ranging from 20-1000 nanometers. These nanofibrous membranes can have surface areas approximately one to two orders of magnitude higher than those found in continuous films. The association of MREs with high surface area electrospun membranes presents the opportunity for developing both novel sampling devices that can also be tailored to fit biosensor detection platforms. It is expected that the available surface area demonstrated by this technique will provide a one to two log increase in sensitivity and capture efficiency, and a less than two-hour response time needed in sensing applications.

Two types of electrospun capture membranes were fabricated containing either carboxyl (COOH) or amine (NH2) functional groups for attachment of antibodies. The carboxylated electrospun polymer used in this study was polyvinyl chloride formulated to be 1.8% carboxylated. The amine functional membrane was made by co-electrospinning two polymers water-soluble polyamine and water insoluble polyurethane. In addition, the electrospun membranes can be tailored to contain more amine functionality by increasing the concentration of the polyamine in the spin dope as indicated by fluorescence experiments.

Linking of molecular recognition groups, antibodies, to the carboxylated PVC was performed using established crosslinking chemistries. Antigen/antibody experiments were performed on the electrospun membranes containing primary antibodies covalently attached on the membranes with different secondary antibody specificity. Results showed that electropun membranes, treated with the secondary antibody, reacted only with its complement as indicated with a chemi-luminescent signal versus those membranes having bound a different, or non-complementary primary antibody.

Toxin studies on Staphylococcal enterotoxin B (SEB) were conducted using avidin/biotin chemistries on the electrospun membranes. Initial experiments on electrospun membranes containing the avidin functional group were performed using a sandwich assay (avidin-SEB biotinylated antibody –SEB toxin- SEB antibody-

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14. ABSTRACT

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HRP). Results showed that 1ng-100ng /ml concentration of toxin were detected in a non-optimized experiment.

Functionalization of the surface of electrospun fibers provides extensive possibilities in designing the high surface membranes with a variety biological capture and detection possibilities to include the multiplexing capabilities by arranging multiple molecular recognition elements on a single electrospun membrane. Exposure of electrospun capture membranes to larger sample volumes provides potential for increasing the sensitivity for detection of biological agents when present in very low or dilute concentrations as might be found in food detection scenarios.

1. INTRODUCTION

The complexity of food matrices can interfere with recovery of the target organisms, as well as the chemistries and signal transduction associated rapid detection technologies. Therefore, appropriate food sampling procedures must be developed and integrated with novel biosensor technologies to provide rapid and accurate tests that will ensure a safe food supply. The type of food being analyzed, e.g. fruits vs. vegetables or smooth surface vs. leafy surface, and pH of food matrix in the extraction solution can affect the attachment, release and recovery of pathogenic organisms (Burnett and Beuchat, 2001). It is well known that traditional culturing processes are time consuming and may underestimate actual numbers of organisms present (Mossell et al., 1995). More rapid detection methods have been developed to compete with the traditional culture methods. These systems, although much faster, generally require a 24 hour enrichment period (Pyle et al., 1999). PCR is considered to be a very sensitive procedure (i.e. rapid system) that offers high specificity and accuracy for pathogen detection. Although there have been claims that PCR has the capability of detecting as low as 1 cfu/ml, this has yet to be reached in food sampling. PCR protocols still require at least an 8-hour enrichment step to ensure specificity and sensitivity due to potential interfering particles in food samples (Lofstrom et al., 2004; Hsu and Tsen, 2001; Zindulis, 2002; Lewis Jr, 2002; McKillip and Drake, 2000).

The usual extraction process for the detection of pathogens from foods involves taking a 25-gram sample and either blending or stomaching the sample in a volume of buffer 10 times greater than the original food sample (Bacteriological Analytical Manual, 1998). This original step results in a 10-fold dilution of the original pathogen concentration. Researchers have been looking for ways to reduce this dilution effect. Direct filtration can result in bacterial entrapment and the filter being clogged by the food particles present. Researchers have been looking at ways to reduce the number of particulates in the homogenized sample and to reconcentrate the bacteria for detection (Kang et al., 2001; Sharpe et al., 2000). Magnetic beads are another capture technology that has received wide use in food sampling due to the ability to capture and concentrate microorganisms, but still requires an enrichment step of 8 to 24 hours for low level pathogen detection (Hotl et al., 1995)]. The magnetic beads may have inherent problems due to low surface area to antibody ratio and susceptibility to non-specific binding. The nanofibrous membranes technology presented in this paper, has an increased surface area allowing for more antibody attachment, can be designed into many different sizes and configurations and is much easier and faster to process. These properties may improve upon current technologies for the capture of low pathogen levels from foods.

Electrospinning is a simple technique that allows for the generation of high surface area membranes using large static electric potentials. This technique has been studied since the 1930's (Formhals, 1934) and has been used to make nanofibers of polymers such as polyethylene oxide, polyacrylonitrile, collagen and even calf thymus DNA (Doshi, 1994; Srinivasan and Reneker, 1995; Fang and Reneker, 1997). In the process of electrospinning the polymer is in the form of either a solution or a melt. A polymer in solution is placed in the proximity of a charged electrode from a DC power source such that the polymer present in the solution/melt becomes charged. At a critical potential, the binding forces with the solution are overcome and a fine jet sprays from the solution surface to the nearest electrically grounded target (Figure 1). As the jet travels to the grounded target the solvent evaporates and the charge on the remaining jet repels itself to split the jet into finer fibers ultimately creating fibers of nanoscale diameter.

2. METHODOLOGY

The electrospinning apparatus used consisted of a DC power source (Gamma High Voltage Research, Inc.

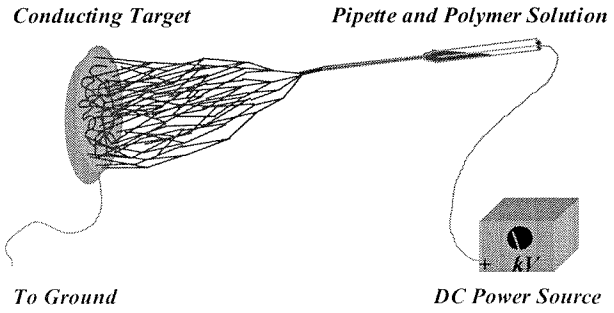

Fig. 1 Diagram showing electrospinning

Model HV ES 30P/100) where the charged electrode wire was immersed in a polymer solution. The polymer solution was drawn into a disposable glass pipette positioned a few degrees down from horizontal. A second ground wire from the power source was attached to a conducting target where the fibers were collected. The electrospinning target used in making the sensing platform was a stainless steel screen in which an electrospun fibrous coating was applied (Fig. 2).

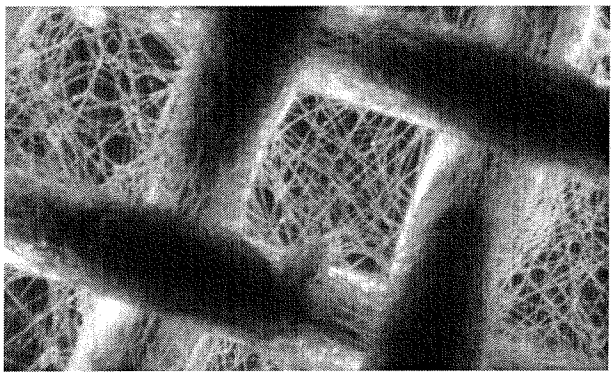

Fig..2 Electrospun membrane shown on a stainless steel screen as seen with a microscope under polarizing light.

2.1 Polymer Solutions for Electrospinning

Membranes containing either the carboxyl (COOH) or amine (NH2) functional groups were fabricated for attachment of antibodies. The aminated electrospun membrane was made by the co-electrospinning of two polymers, polyamine and polyurethane. The PAA-H-10C (poly(2-propen-1-amine), Nitto Boseki, LTD) 10% solution in water was used as the amine fraction of the co-polymeric membrane. Thirty to 150 milligrams of the PAA-H-10C was dried down in a vacuum to a concentrated solution for inclusion in the spin dope prior to electrospinning. The polyurethane used was Pellethane 80AE (Dow Chemical, Midland, MI) was solubilized 10% by weight in DMF and the fraction of the PAA-H-10C was added with vigorous stirring keeping both polymers in solution. The membranes were electrospun quickly to avoid precipitation by either the polyamine or polyurethane. Electrospinning was conducted for approximately 10 minutes on the target screen. After electrospinning, the screens were cut with a dye punch to uniform sizes to minimize size and weight variances. The polyurethane/polyamine membranes combining both a water-soluble and water insoluble polymer were tested for amine activity. The membranes were first dialyzed in water for 24 hours to remove unincorporated water-soluble amine polymer. Labeling remaining incorporated with the amines in the fiber was determined by using Fluorescein-EX kit protein labeling kit (Molecular Probes, Eugene, OR). The fluorescent signal was detected by using blue laser detection mode on the STORM 860 (Molecular Dynamics, Sunnyvale, CA).

The carboxylated polymer used to fabricate electrospun membranes was polyvinyl chloride 1.8% carboxylated (PVC-COOH) (Aldrich Chemical, St. Louis, MO). This polymer was solubilized at 10% by weight in 80% N,N-dimethyl formamide (DMF) and 10% tetrahydrofuran (THF). Two crosslinking chemistries were studied for antibody attachment to the electrospun membrane. One process linked the antibodies to the carboxylated PVC using N-Hydroxysulfo-succinimide (Sulfo-NHS) and the dehydrating agent 1-ethyl-3-(3-Dimethylaminopropyl) carbodiimide Hydrochloride (EDC) (Pierce Chemical Company, Rockford, IL). In this procedure the membrane was treated as a carboxylated protein to be linked with an antibody. The second crosslinking chemistry studied used avidin and fluorescein labeled biotin. The avidin was attached on the PVC-COOH membranes using EDC carbodiimide (Pierce Biotechnology, Rockford, IL) covalent chemistry. Again, the membrane was treated as the initial carboxylated protein in one milliliter of reaction buffer pH 5.0 MES/0.1% tween 20. The second protein, avidin, was added at a concentration of 0.5mg/ml to the reaction buffer to which 1mg of EDC was added to initiate the conjugation reaction to the membrane. The carboxylated membrane and avidin reacted two hours at room temperature. After the conjugation reaction the membranes were washed 3X with pH 7.2 PBS/0.1%tween 20 (PBST) to allow buffer equilibration with gentle rocking. Biotin-fluorescein conjugate was added to the membranes 0.5 mg/ml in the same buffer and allowed to react for 1 hour. The membranes were then washed again 3X with the PBS buffer and the fluorescent signal was detected by using blue laser detection mode on the STORM 860.

2.2 Capture Studies

Antigen/antibody experiments were performed to determine the potential for producing electrospun membranes with capture specificity. Two different primary antibodies were used for the experiment, Rabbit anti-*Staph* and Goat anti-Rat, were covalently attached to two separate electrospun membranes using the EDC and Sulfo-NHS crosslinking chemistries above. The same secondary antibody, Rabbit anti-Goat- HRP conjugate was used for the antigen/antibody specificity assay (Goat anti-Rat/Rabbit anti-Goat HRP conjugate). The chemiluminescent signal was produced using Super Signal (Pierce Chemical Company) detected on the SynGene CHEMIGENIUS 2 Bio-imaging system. The samples were done in duplicate.

Experiments were conducted to detect Staph Entero toxin B (SEB) using electrospun PVC-COOH membranes

with attached anti-SEB antibodies to capture and concentrate the toxin. Highly purified SEB, Rabbit anti-SEB biotinylated and HRP antibodies (Toxin Technology, Sarasota, FL) were used for this study. Biotinylated Rabbit anti-SEB was attached to the avidin crosslinked membrane in a 1/2000 dilution for 1 hour at room temperature with gentile shaking. The membrane was then blocked with 0.2% non-fat dry milk (NFDM) in PBST. NFDM blocked electrospun PVC-COOH membranes without antibodies were used as controls to determine extent of non-specific binding in the experiment. Membranes with and without Biotinylated SEB antibodies were reacted with SEB concentrations in PBST at 0, 1,10, and 100 nanograms of SEB toxin per ml for 1 hour with gentile shaking in 24 well polystyrene titer plates (Dow Corning, Midland MI). All experimental and control samples were done in triplicate. The membranes were washed 5X with 1 ml PBST with slightly vigorous shaking for 5 minutes on a LabLine Microtiter plate shaker Model 4265. The membranes were then reacted with a 1/10000 dilution of SEB-HRP conjugate antibody in 1 ml PBST for 1 hour with gentile shaking. After binding the membranes were then washed 5X with 1 ml PBST with shaking to remove excess labeled antibody. The membranes were then removed from the 24 well titer plates and placed into individual wells of a 96 well titer plate to which 100 microliters of Super Signal reaction mixture was added for detection of chemiluminescent signal. Light generated from the peroxides-chemiluminescent reaction was collected by positioning a fiber optic probe above the membrane surface in the absence of light. The probe was connected to a A5-2021A Lumi-Tec luminometer (ST. Johns Associates, Beltsville, MD) for measurement of chemiluminescence.

3. RESULTS AND DISCUSSION

3.1 Aminated Electrospun Membranes

Results from aminated Pellethane electrospun membranes are shown in Figure 3. The amines associated with the membranes were labeled with fluorescein isothiocyanate and monitored over time for relative amounts of amines present. The graph shows fluorescein counts corresponding to overall concentrations of polyamine co-electrospun with the Pellethane polymer. Due to water solubility of the polyamine polymer, there was concern that the amines would not be part of the membrane under aqueous conditions needed for covalent molecular recognition attachment. The membrane was washed with water over time and the fluorescein labeled amines associated with the nanofibrous membrane were monitored. Results show that fluorescein counts differed significantly with the amounts of polyamine added to the initial spin dope solution prior to electrospinning. Washing over time did seem to have a small effect for removal of fluorescein but the majority of initial amine concentration was still present. These results show ability to control the amount of functional groups within the electrospun fibrous membrane by varying the initial concentration of the amine polymer added. This could allow one to "tailor" the process of membrane fabrication

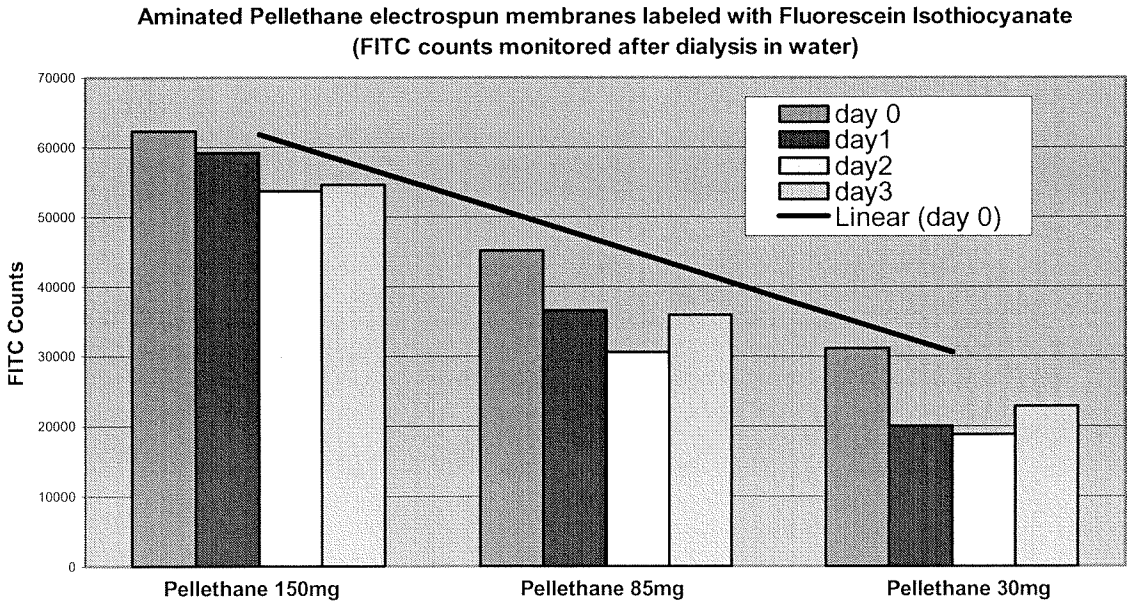

Fig. 3 Shows graph of fluorescein labeled free amines associated with the electrospun membrane. Membranes were washed with MQ water and measured on the STORM 860 for fluorescence. Milligram amounts correspond to the dry weight of the polyamine polymer added to a 10% Pellethane spin dope prior to electrospinning.

according to what is needed for biomolecule attachment.

3.2 Carboxylated Electrospun Membranes

The PVC-COOH polymer was studied due to the known presence of carboxyl groups available for attachment of molecular recognition chemistries. These membranes with specific antibodies attached could then be used to demonstrate the capture and concentration concept in the electrospun membrane configuration described in this paper. Figure 4 illustrates the antigen/antibody experiment that was performed on the electrospun membranes to demonstrate the capture specificity potential of the membrane. Different primary antibodies were attached by EDC and Sulfo-NHS

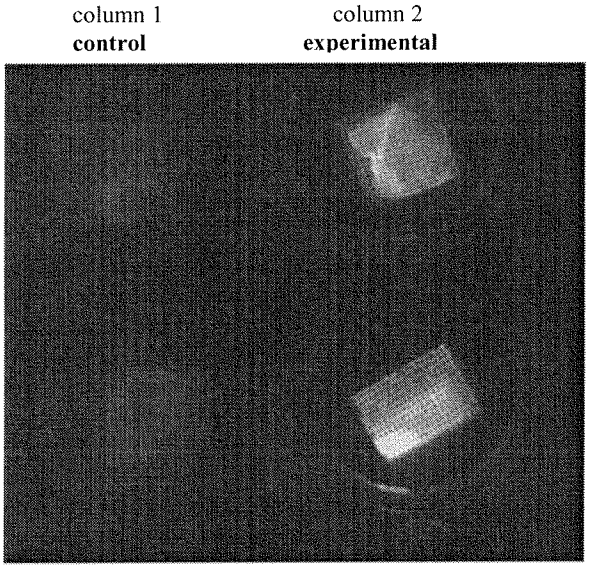

Fig. 4 Crosslinkers: EDC and Sulfo-NHS; **Control: 1° antibody**= Rabbit anti- *Staph*; **Experimental**: 1° **antibody**=Goat anti-Rat 2°antibody=**Rabbit anti-Goat HRP** conjugate

crosslinkers to separate membranes. The carboxylated membranes possessing the different primary antibodies were then both reacted in exactly the same manner to the secondary HRP labeled antibody. In column 1 are the results of the electrospun membranes to which Rabbit anti-*Staph* antibody was attached. This antibody was used as the control since it is not the complement to the secondary antibody. As would be expected, there was only a slight chemiluminescent signal produced which may have been due to non-specific binding of the antibody to the membrane. In contrast to the control, column 2 shows the results of the membrane to which Goat anti-Rat, the complement to the secondary labeled antibody, was used as primary capture antibody. One can see that the membranes treated with Goat anti-Rat and its complement Rabbit anti-Goat (HRP) had a strong chemiluminescent signal produced. The results from this experiment demonstrated the capability of utilizing the electrospun membrane architecture for specifically capturing antigens from solution.

Shown in Figure 5 are the results of the avidin/biotin binding studies conducted on PVC-COOH electrospun membranes. The results indicate that the biotin-fluorescein conjugate does not strongly associate when

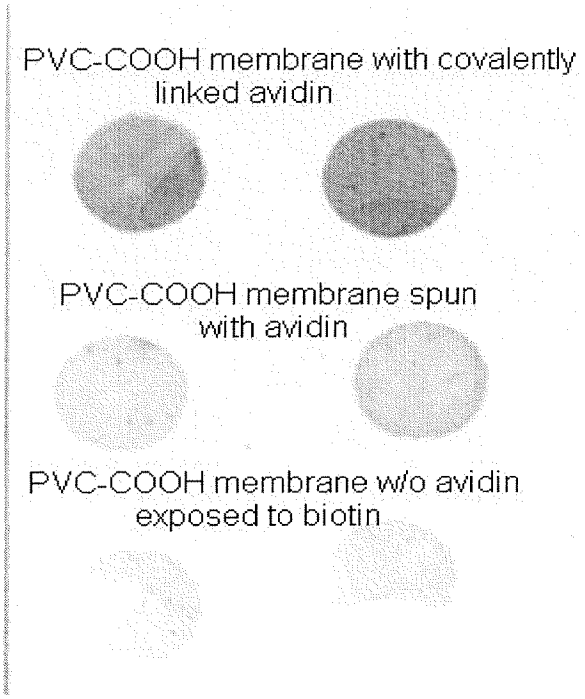

Fig. 5 Avidin/biotin studies conducted on PVC-COOH electrospun membranes

avidin is not chemically crosslinked to the electrospun membranes. In addition, the biotin-fluorescein molecule does not adsorb or "stick" by itself to the membrane as well. This indicates that little or no nonspecific binding is occurring with the avidin/biotin coupling strategy. With this result in mind we adopted the biotin/avidin scenario for the SEB toxin study looking to minimize the occurrence of nonspecific binding.

The PVC-COOH membranes used in the SEB sandwich immunoassay study were produced at a diameter to fit the well of a standard 96 titer plate. One set of membranes had avidin chemically crosslinked to the membrane coupled with biotinylated anti-staph antibodies. The second set of membranes used in this study were electrospun PVC-COOH without avidin and the biotinylated antibody. Both membrane sets were

reacted in similar fashion. The controls for both membrane sets were only exposed to the anti-staph HRP labeled antibodies. All test samples were first reacted with known concentrations of SEB and then the secondary labeled antibody. The data in Table 1, demonstrate that the membranes could capture a 1- ng/ml concentration of SEB under the current assay conditions, which have not been optimized. The test was designed to see if the electrospun membranes with antibodies specific for SEB could specifically capture and detect by chemiluminescence one of the SEB concentrations. It was interesting to learn that the membrane configuration could detect by a factor of greater than 2 the lowest 1 ng/ml concentration, demonstrating the potential sensitivity of the membrane capture system.

Table 1. Membranes with avidin linker and biotinylated antibody

Ng/ml SEB	Avg. Chemilum.	Std. Dev.	LOD Chemilum.	Detection of SEB
0	0.67	0.25	**1.17**	
1.0	2.47	0.99		Yes
10	10.63	3.47		Yes
100	96.23	23.19		Yes

Another important detail identified in the study was that both the toxin and the secondary antibody did not bind non-specifically to the high surface electrospun membrane, Table 2. Even at the highest concentration of 100 ng/ml there was no chemiluminescent signal over background produced by the membranes without primary antibodies. This showed that the primary antibody was necessary to first capture the SEB in order for a signal to be generated.

Table 2. Membranes without avidin linker and biotinylated antibody

Ng/ml SEB	Avg. Chemilum.	Std. Dev.	LOD Chemilum.	Detection of SEB
0	0.73	0.57	**1.30**	
1.0	0.86	0.06		No
10	0.30	0.26		No
100	0.13	0.06		No

4. CONCLUSION

A nano-fibrous membrane concept was developed to improve sampling processes for food and water monitoring to specifically concentrate and detect the analyte of interest from extracted and dilute samples. Generating high surface area membranes through the use of electrospinning, unique polymeric membranes were formulated with definite functionalities that will allow covalent attachment of molecular recognition elements (e.g. antibodies, aptamers, peptides, PNAs (peptide nucleic acids), liposaccharides, etc.) for the selective capture of target analytes. This methodology could increase the probability of finding and detecting analytes present at low concentrations, such as bacterial pathogens or toxins. Technologies that improve both the capture and pre-concentration of target analytes from food matrices and environmental samples prior to detection are needed to insure that cells and toxins have been collected and can be detected and identified. Increasing surface area for capture of both chemical toxins and biological organisms could improve the sensitivity needed for sensor development. Cast films versus electrospun membranes of the same polymer were compared for reactivity. Results indicated electrospun membranes were far more reactive than the cast films indicating reduced functional groups available for attachment in the polymeric films (data not shown).

Studies shown in this paper indicate polyurethane membranes can be tailored to include water-soluble polyamine, providing the necessary functional primary NH_2 for attachment of biomolecules. Fluorescence measurements show primary amines can be added in a concentration dependent manner to the spin dope prior to membrane formation. Electrospun membranes of polyvinyl chloride (PVC-COOH) containing carboxyl groups for attachment of biomolecules, through covalent chemistries to include antibodies and the avidin/biotin coupling strategy, have shown conclusively that sensor elements can be linked to the membrane and that one can design the membranes according to detection needs. Immunoassays using antibodies demonstrated potential for antigen specificity. The avidin/biotin strategy used for toxin studies further demonstrated the actual capture of toxin, down to the 1 nanogram level, without optimization. This is the first demonstration to our knowledge that electrospun polymeric membranes coupled with biomolecules have shown potential as platforms for biological/chemical sensing.

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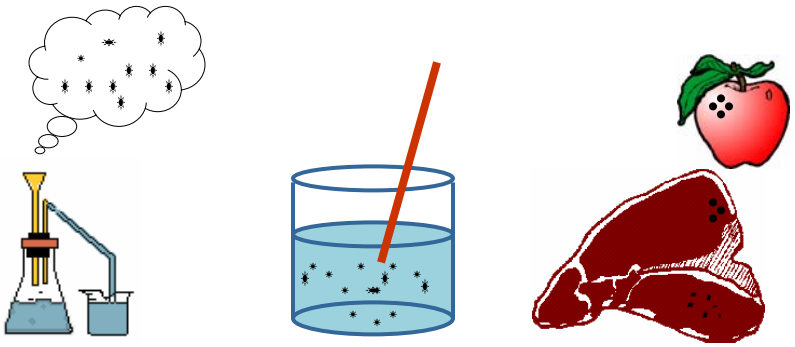
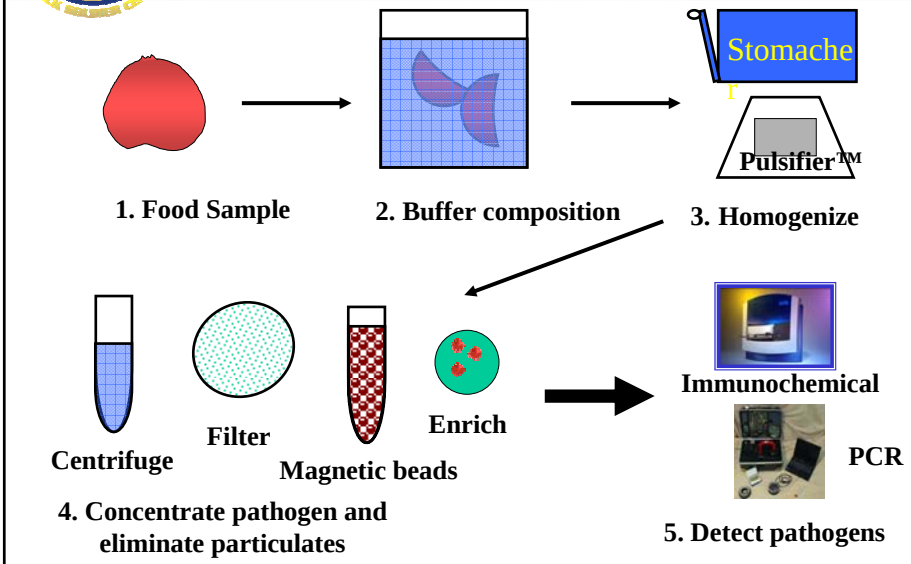
Doing something for the Soldier for the past 50 years…

Pathogen Sampling

Air vs. **Water** vs. **Food**



Pathogen Sampling



Department of Defense COMBAT FEEDING

Nanofiberous Membranes

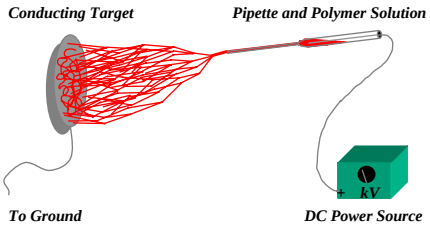
The goal was to develop a novel sampling technology using electrospun nanofiber membranes associated with specific capture chemistries that could lead to improved detection capabilities for low concentrations of pathogens and toxins from complex and dilute samples by improving the capture and concentration step in the sampling process.

Department of Defense COMBAT FEEDING

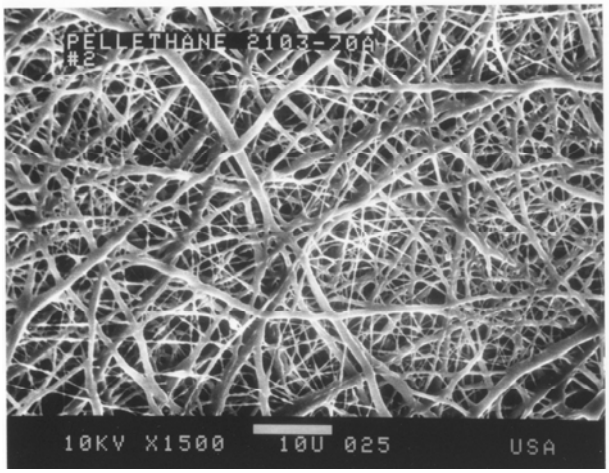
Nanofibrous Membranes

Why Electrospinning?

- Vary fiber diameter
  - Solution viscosity
  - Field strength
  - Net charge density in fiber
  - Solvent evaporation rate
  - Polymer molecular weight
- Ultra Thin (conformable)
- High Surface Area
- High Porosity (breathable, lightweight)
- Membrane-like Textile
- Spin into air, vacuum, other gases
- Spin from dilute *solution*, or *melt*
- Co-spinning of polymers/additives
- Simple Manufacturing/Custom Design Tailoring

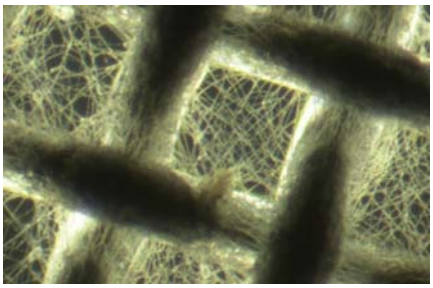


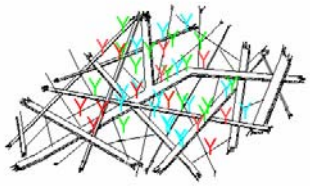
SEM Micrograph



Nanofibrous Membranes

Envisioned Membrane with Molecular Recognition Element (MRE) Attachment





Electrospun membrane containing capture elements, in this case antibodies. Note increased amounts of MRE;s possible with this multidimensional technology

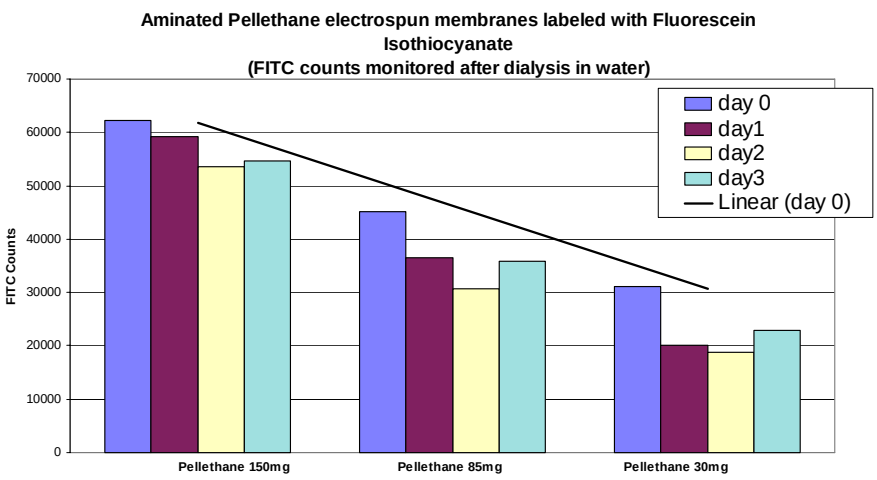
Materials and Methods:

Membranes with NH_2 functional groups:

Electrospun polyurethane: Pellethane Dow Chemical Additive consisted of : polyamine to give primary NH_2 group attachment.

Fluorescein isothiocyanate (FITC) succinimidyl ester binding to primary amines on fiber surface.

Able to determine primary amines using Sulfo-SDTB reaction.



Materials and Methods

Carboxylated PVC Membranes:

Electrospun material: Polyvinyl Chloride-COOH (1.8%).

Antibody/Antigen test: Goat anti rat IgG, IgM and rabbit anti goat IgG (HRP) activity tested with SuperSignal ELISA Pico (Pierce).

SEB Toxin Capture/Detection Studies: Avidin Crosslinked Membranes with biotinylated rabbit anti-SEB antibody

Sandwich Immunoassays were performed with SEB at various concentrations with HRP conjugated antibodies

Antigen Binding

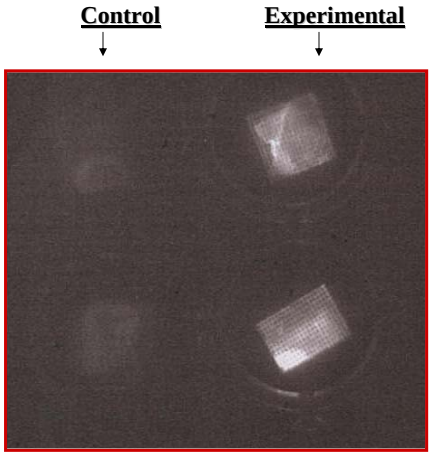
Antibody/Antigen test on PVC-COOH membrane specificity

Crosslinkers: EDC and Sulfo-NHS

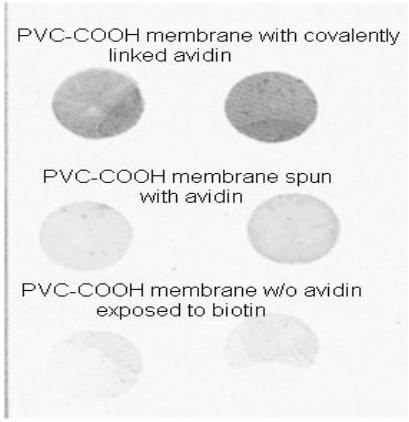
Control: 1˚ antibody= Rabbit anti-Staph

Experimental: 1˚ antibody= Goat anti-Rat

2˚antibody=Rabbit anti-Goat HRP

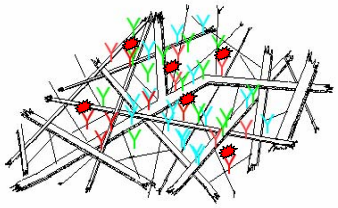


Avidin/biotin (fluorescent) studies conducted on PVC-COOH electrospun membranes



SEB Assay

Antibody capture of (SEB) on a nanofiber membrane



Staphylococcal enterotoxin B detected with horseradish peroxidase-antibody conjugate

Y Capture Antibodies

Department of Defense COMBAT FEEDING

Nanofiber Membrane Results

	Membranes with avidin linker and biotinylated antibody			Membranes without avidin linker and biotinylated antibody	
ng/ml SEB	Chemiluminescent Signal	Detect SEB		Chemiluminescent Signal	Detect SEB
0	0.67			0.73	
1.0	2.47	Yes		0.86	No
10	10.63	Yes		0.30	No
100	96.23	Yes		0.13	No

Department of Defense COMBAT FEEDING

Summary of Results

- **Polyurethane membranes can be co-electrospun together with a water soluble polyamine to insert primary NH_2 groups for attachment of reactive compounds.**
- **Fluorescent compounds that bind to the primary NH_2 bind to the membranes in a concentration dependent manner to polyamine addition in the spin dope.**

Summary of Results

- **Electrospun membranes Polyvinyl chloride (PVC) membranes containing COOH reactive groups.**
- **Reacted carboxylated PVC membranes for attachment of antibodies to the electrospun membranes. Conducted immunoassay of antibodies indicating specificity with its complement.**
- **Avidin/Biotin linked membranes detected SEB toxin in a immuno-sandwich assay at concentrations of at least 1 ng/ml.**

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